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Observations on the breeding biology of Collembola (I)

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With 5 figures in the text
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1. Introduction

Studies on the breeding biology of Collembola can be divided into two separate groupings: single-genus or single-species studies, in some cases with economic considerations, and comparative accounts of reproduction and behaviour of Collembola in general. So far little attention has been paid to the life-cycles of moorland Collembola, and the present work provides information on the breeding biology of some Collembola, with particular reference to the high moor fauna of the Moor House National Nature Reserve, Westmorland (2000 ft. 610 m O. D.).

The species on which observations were made are as follows:

- Hypogastrura denticulata* (BAGNALL, 1941) sensu GISIN, 1949
- Neanura muscorum* (TEMPLETON, 1835)
- Onychiurus furcifer* BÖRNER, 1901
- Onychiurus procampatus* GISIN, 1956
- Onychiurus latus* GISIN, 1956
- Onychiurus tricampatus* GISIN, 1956
- Tullbergia krausbaueri* (BÖRNER, 1901)
- Isotoma sensibilis* (TULLBERG, 1867)
- Isotoma viridis* BOURLET, 1839
- Isotoma olivacea* TULLBERG, 1871
- Isotoma infusata* (MURPHY, 1959)
- Isolomurus palustris* (MÜLLER, 1776)
- Lepidocyrtus lanuginosus* (GMELIN, 1788)
- Tomocerus minor* (LUBBOCK, 1862)
- Dicyrtoma minuta* (O. FABRICIUS, 1783)
- Dicyrtoma fusca* (LUCAS, 1842)

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With the exception of *Onychiurus jureijer*, which was collected at Hamsterley, County Durham, all the Collembola cultured were obtained from Moor House.

2. Methods

Litter containing Collembola was brought back to the laboratory, and the animals extracted onto the surface of water using a Tullgren funnel. Collembola were removed from the water surface and introduced into cultures using a piece of fine tinned copper fuse wire which had previously been heated and flattened and then mounted in a glass rod.

A method of culturing similar to that described by GOTO (1961) was found to be most satisfactory, and culture vessels were made as follows: 5 cm lengths of Pyrex glass tubing 5 cm in diameter were used, and these were embedded in a mixture of plaster of Paris and powdered charcoal retained in an aluminium lid 6 cm in diameter. On drying, the gap between the aluminium lid and the glass was filled with wax, to prevent evaporation from the plaster block. The container was enclosed by a cork which was covered by a piece of polythene sheet to prevent fungus growing on it. The surface of the plaster was flamed before the introduction of Collembola, and distilled water was used at all times to moisten the surface. This procedure kept the cultures free from fungi and bacterial growths, except for those introduced with the food. Moistened yeast pellets were found to be satisfactory material on which to feed the Collembola, provided that the cultures were examined regularly each day; fungal mycelia sometimes grew out from the yeast over the sterilised substrate, and when this happened the yeast was brushed to remove it.

Individual Collembola were cultured in $2 \times \frac{3}{4}$ inch (2×1.9 cm) specimen tubes filled to within $\frac{1}{2}$ inch (ca. 1.3 cm) of the top with a plaster of Paris and charcoal mixture, and covered with a $\frac{3}{4}$ inch (1.9 cm) diameter cover slip retained by a film of vaseline. Eggs were also kept in tubes of this type, but these were covered by polythene bungs.

Cultures were kept at constant temperatures by enclosing the culture vessels in large jars which were submerged in tanks of water where the temperature was maintained by means of a thermostat. Except where otherwise stated individuals were reared at 8 °C.

Nomenclature of Collembola follows GISEN (1960).

3. The spermatophores

SCHALLER (1953) describes the spermatophores of *Orchesella villosa* (GEOFFROY, 1764) and this description generally applies to most species of Collembola so far examined. During the course of this work the spermatophores of two species, *Tomocerus minor* and *Dicyrtoma minuta* were regularly encountered. Those of *Tomocerus minor* possessed a stalk of average length $265.5 \mu\text{m}$ (S. E. ± 1.6) and had a sperm droplet of diameter $54.7 \mu\text{m}$ (S. E. ± 1.1). The spermatophore in *Dicyrtoma minuta* was larger; this had an average stalk length of $696.0 \mu\text{m}$ (S. E. ± 2.9) and a sperm droplet of average diameter $96.2 \mu\text{m}$ (S. E. ± 2.8). Ten spermatophores of each species were measured. The diameter of the sperm droplet varies with the relative humidity, and the measurements in the present work were made at 100% R. H. The measurements given by MAYER (1957) for the diameter of the sperm droplet of *Dicyrtoma minuta* ($49\text{--}55 \mu\text{m}$) were probably made in an R. H. below 100%, under which circumstances the droplet is smaller.

Whilst SCHALLER (1953) makes no mention of the structures which maintain the sperm droplet at the top of the stalk of the spermatophore, MAYER (1957) describes a double branching in the stalk of the spermatophore of *Orchesella villosa*, and illustrates this together with the cone-shaped structure occurring at the top of the spermatophore stalks in *Sminthurus fuscus* (LINNÉ, 1758). In the two species examined by the present writer, each maintained the droplet at the top of the stalk by different methods. In *Tomocerus minor* the top of the stalk is curved over into a circle and the sperm droplet hangs in it or round it; in *Dicyrtoma minuta* the tip of the stalk carries a broader cone, the apex of which projects through the sperm droplet which is maintained at the top of the stalk by the broader base of the cone (Fig. 1), as in *Sminthurus fuscus*.

SCHALLER (1953) records that the sperms are retained within a pellicle on the stalks, and this is also the case in *Tomocerus minor* and *Dicyrtoma minuta*. However, it was found

that in both cases the pellicle did not break on contact with water, as was recorded by both SCHALLER (1953) and MAYER (1957). The pellicle was found to be remarkably resistant and the whole spermatophore could be mounted in water, lacto-phenol or poly-vinyl-lacto-phenol, without bursting. These observations suggest that it is only by mechanical fracture that the pellicle of the sperm globule can be broken.

In *Dicyrtoma minuta*, MAYER (1957) found an average of fifty spermatophores per male at each deposition, with a maximum of sixty-nine; this compares with two counts of fifty-three and fifty-five in the present work. No estimate was possible in *Tomocerus minor* where spermatophores were produced in mass cultures.

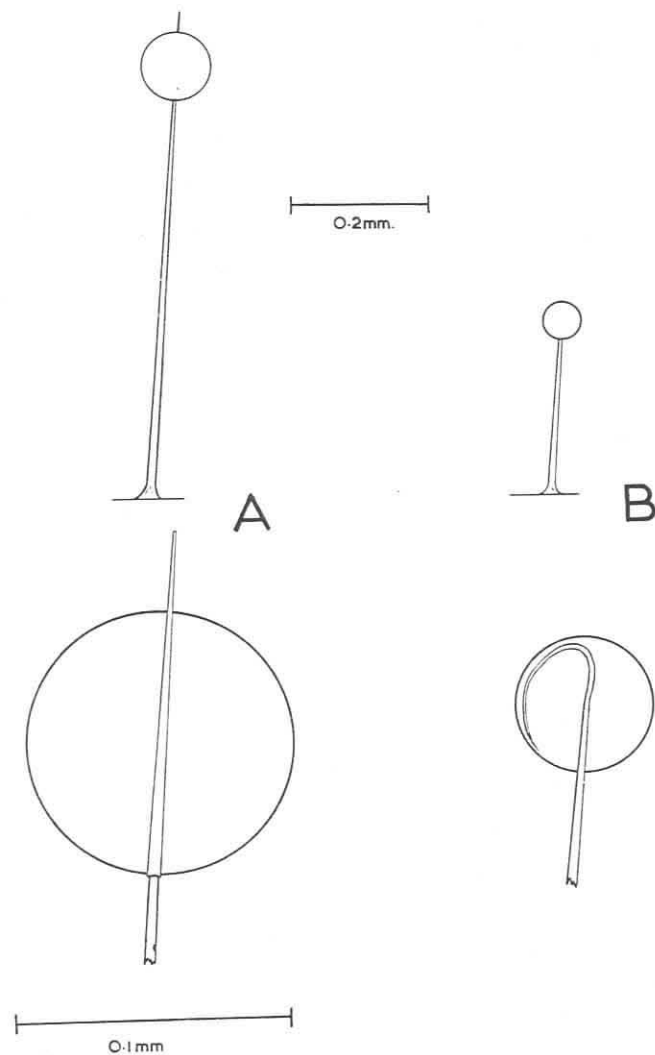


Fig. 1. Spermatophores of *Dicyrtoma minuta* (A) and *Tomocerus minor* (B). Top: Appearance of newly deposited spermatophore; Bottom: Detail of the support of the sperm droplet.

4. The transfer of sperms

In *Orchesella villosa* and *Tomocerus vulgaris* (TULLBERG, 1871) the females strip off the sperm droplets from the stalked spermatophores at random during movement, and a droplet of liquid is exuded from the genital aperture of the female, into which the sperm droplet supposedly bursts (SCHALLER, 1953). In the light of the present observations concerning the resistance of the pellicle, this seems unlikely to occur in the species covered in this paper, and the sperm droplets probably burst on contact with the cuticle of the female.

In an attempt to cast some light on the problem of the uptake of the sperm by the females, the following experiment was carried out. Twelve female *Dicyrtoma minuta* were isolated singly in culture tubes, and another twelve tubes were prepared, each of which contained a male. None of the females produced eggs, and after 40 days it was assumed that they had not been previously fertilised. At this time two of the males had produced 53 and 55 spermatophores respectively, and after removing the male from each of these tubes a single female was introduced. The females were allowed to remain in the spermatophore-containing tubes for a period of five minutes, during which time they were continuously observed. Neither individual appeared to appreciate the presence of spermatophores, and ran about the tube brushing against them with the underside of the body and legs. In this way several sperm droplets were caused to burst on the body surface, purely by mechanical contact; no droplet was observed to be exuded from the genital aperture of the females, as recorded by SCHALLER (1953). After a period of five minutes the two females were returned to their own tubes, where after 4 days they produced eggs which eventually hatched. None of the other isolated females produced eggs. The period between contacting spermatophores and oviposition is comparable with that observed by SCHALLER (1953) in *Orchesella villosa*, of three hours to three days.

Once on the body surface of the female, the sperms may travel to the genital aperture by a chemotactic response, or they may be purposively transferred there. The only structure which seems capable of reaching most parts of the body of *Dicyrtoma minuta* is the filament of the ventral tube (see NUTMAN, 1941); when extruded each filament reaches a length of almost twice that of the body. If the ventral tube is used in sperm collection, then the records of individuals taking sperm into the mouth, i.e. LEMOINE (1882), LIE-PETERSEN (1899), STREBEL (in SCHALLER 1953), suggest the use of the ventral groove to pass the sperm to the filaments of the ventral tube. The ventral groove arises just behind the labium (HOFFMAN, 1904, RUPPEL, 1953) and secretions of the cephalic gland are passed along it to the ventral tube. The filament of the ventral tube could be used to introduce the sperm to the genital aperture. As yet this is purely surmise, but it is a possibility worthy of future consideration.

In *Podura aquatica* LINNÉ, 1758 and *Hypogastrura armata* (NICOLET, 1841) apparently no spermatophores are produced but the sperm droplets are deposited on the substrate, and the female gathers the sperm by rubbing the genital plate over the droplet (STREBEL, 1932; BRITT, 1951). In neither case was the presence of sperm in the sperm droplet verified. The possibility of direct transmission of sperm by the placing together of the genital plates of the male and female in *Archisotoma besselsi* (PACKARD, 1877), as described by WILLEM (1925), is supported by the present writer in the case of *Onychiurus tricampatus* and *Onychiurus jureijer*. In two observations involving these two species, males were observed in cavities in the substrate of the culture jar (caused by the formation of air bubbles as the plaster of Paris set) in the presence of another individual of the same species. In both cases a droplet of sperm was hanging from the genital plate (removed and examined by microscope). No definite pellicle was present over the droplet, and motile sperms were found to be present in both cases. Movement of either individual in the

confined space would have resulted in the sperm droplet bursting upon the other individual. The sex of the second individual was in neither case determined. MAYER (1957) records that in *Onychiurus armatus* TULLBERG, 1869 (not sensu GISIN, 1952) and *Tullbergia quadrispina* (BÖRNER, 1901) transfer of sperms occurs by the females picking up drops by means of the genital plate, deposition and acceptance of sperms being undirected by the other partner.

Sperm transfer in at least some of the Poduridae and Onychiuridae appears to be accomplished by the introduction of free sperm into the genital aperture of the female, either by collection from the substrate with no part being played by the male, or by the female brushing it off the genital plate of the male when in close proximity to it. No definite information exists for the Isotomidae although the observations of WILLEM (1925) on *Archisotoma besselsi* suggest a transfer of sperm with the male taking an active part. In the Entomobryidae spermatophores occur, the sperm being taken up during random movement of the female. In the Sminthuridae spermatophores exist in the genera *Sminthurinus*, *Sminthurus* and *Dicyrtoma*, and possibly both deliberate (STREBEL in SCHALLER, 1953, MAYER, 1957) and accidental collection (SCHALLER, 1953, present writer) occur; LIE-PETERSEN's (1899) observations suggest that some species of the Sminthuridae do not possess spermatophores, and this has been confirmed by MAYER (1957).

5. The eggs

5.1. Oviposition

DAVIDSON (1934) records that prior to oviposition in *Sminthurus viridis* (LINNÉ, 1758) a drop of 'anal fluid' is secreted from the anus and this is deposited on the soil surface so that the egg becomes coated with it as it is extruded; this implies that the tip of the abdomen is in contact with the substrate during the extrusion of the egg from the genital aperture. The present writer has observed oviposition in several individuals of the following species in culture: *Hypogastrura denticulata*, *Onychiurus furcifer*, *Onychiurus latus*, *Onychiurus procampatus*, *Onychiurus tricampatus*, *Tullbergia krausbaueri*, *Lepidocyrtus lanuginosus*, *Isotoma sensibilis*, *Dicyrtoma fusca* and *Dicyrtoma minuta*. In all cases the abdomen tip was elevated until the egg was extruded, and special postures were assumed by laying females which apparently had the effect of preventing the egg from contacting the substrate until fully extruded (Fig. 2).

During the process of extruding the egg from the genital aperture, the female normally remained immobile. In *Dicyrtoma fusca* and *Dicyrtoma minuta* the abdomen tip pulsates during the process and particles of soil and faecal material move round the outside of the chorion, bathed in anal fluid; these particles move slightly at each pulsation and originate from the anus. In this way the egg may become entirely covered with dark particles before it is finally deposited. Deposition occurs with an abrupt cessation of the pulsating movement and the abdomen simultaneously drops onto the substrate. The female then rocks from side to side whilst the claws retain their purchase and movement originates at the joints of the tibiotarsi and femurs; the rest of the body remains motionless. The rocking motion is exaggerated anteriorly and this causes a rotation between the genital plate and the egg. The insect breaks free after about five seconds and moves quickly away, picking up material from the substrate and apparently eating it. This activity is reminiscent of the displacement activities of vertebrates (ARMSTRONG, 1950). Only in *Dicyrtoma fusca* and *Dicyrtoma minuta* were the eggs coated with faecal material during the laying process, as DAVIDSON (1934) records in *Sminthurus viridis*.

In the other species observed, the eggs are laid in batches and as an egg is extruded it is deposited on the substrate by a slight jerking action; the next egg is laid from a similar posture, with no contact between the two eggs, and eggs are removed from the genital plate either by subsequent contact with the substrate or with the egg batch. The insects

free themselves from an egg batch simply by walking away from it. Occasionally single eggs were found in the cultures and these were probably due to the female being disturbed whilst laying.

Table 1 shows the times recorded for the production of egg batches in seven species in culture. Continuous observations were made on all except the two species of *Dicyrtoma* which were observed hourly. The two species of *Dicyrtoma*, which lay their eggs singly, take ten times as long as any of the other five species which lay their eggs in batches, to produce a single egg.

The eggs of Collembola are usually laid in batches (PACLT, 1956). In the cultures three species did not lay their eggs in batches. *Tomocerus minor* laid its eggs singly, wherever possible in a crevice the same size as the egg itself. *Dicyrtoma fusca* and *Dicyrtoma minuta* distributed their eggs over the whole of the culture substrate and here again the eggs were placed in crevices and depressions. *Dicyrtoma minuta* deposited its eggs where a pattern of scratches was provided on the surface of the culture medium; all eggs were laid in the grooves. Occasionally in all three species up to three eggs were found together in a cluster, but never more.

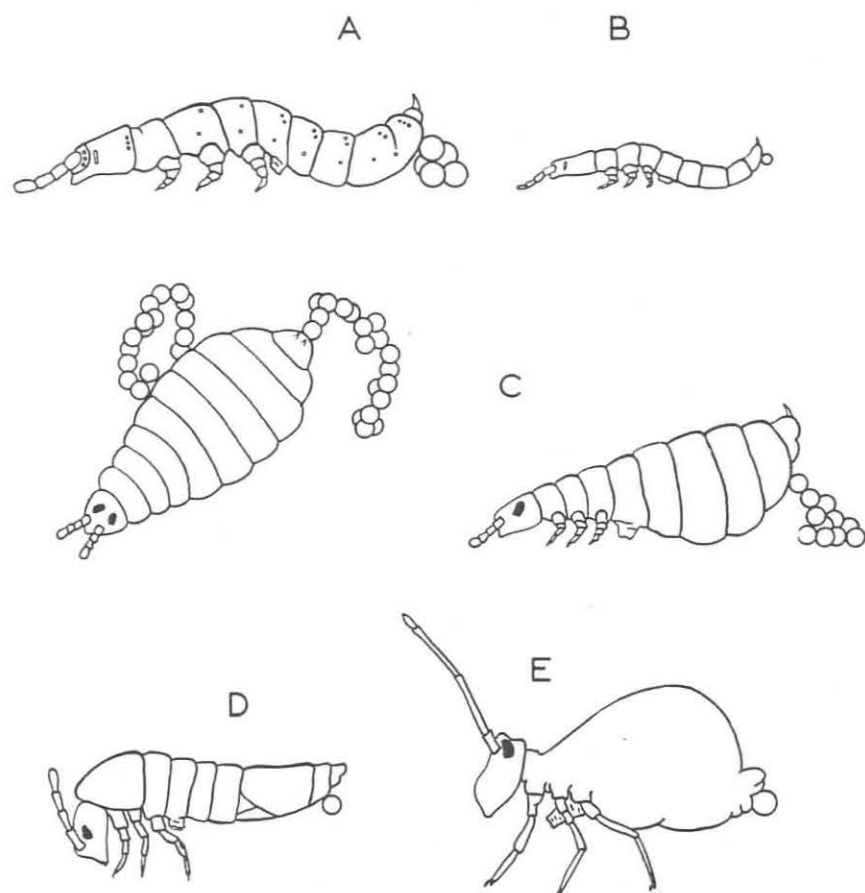


Fig. 2. The postures assumed by female Collembola during oviposition. A. *Onychiurus procamptus*; B. *Tullbergia krausbaueri*; C. *Hypogastrura denticulata*; D. *Lepidocyrtus lanuginosus*; E. *Dicyrtoma minuta*.

Table 1 Duration of oviposition in Collembola.

Species	Batch size	Time taken to lay batch in minutes	Average time to lay one egg in minutes
<i>Hypogastrura denticulata</i>	33	90	2.9
	25	75	
	30	90	
<i>Onychiurus latus</i>	15	40	2.6
	12	30	
<i>Tullbergia krausbaueri</i>	10	35	3.9
	8	35	
<i>Isotoma sensibilis</i>	20	45	2.3
	12	30	
<i>Lepidocyrtus lanuginosus</i>	35	120	3.4
<i>Dicyrtoma minuta</i>	25	1440	55.4
	26	1440	
	27	1440	
	26	1440	
<i>Dicyrtoma fusca</i>	17	1440	79.4
	25	1440	
	18	1440	
	19	1440	
	14	1440	

In the cultured species which lay their eggs in batches, wherever possible the eggs are placed in a cavity. In the plaster of Paris medium, holes left by air bubbles as it sets are a favourite situation for the deposition of eggs, and there is a suggestion that females lay more readily in culture jars containing such cavities.

5.2. The newly laid eggs

PACLT (1956) states that the newly laid eggs of Collembola are shining white; this is not invariably the case as MILNE (1960) found. In the present work it was observed that the eggs of *Isotoma viridis*, *Isotoma sensibilis* and *Tomocerus minor* are orange when first laid, and later take on a brownish tinge, whereas the eggs of *Neanura muscorum* are a characteristic delicate shade of cream tinged with pink. The eggs of *Hypogastrura denticulata* are like small pearls when first laid and the eggs of *Onychiurus latus*, *Onychiurus tricampatus* and *Onychiurus procampatus* are similar but have a slightly whiter appearance and are less translucent. The eggs of *Isotoma olivacea* and *Isotoma infuscata* are almost transparent on laying whereas the eggs of *Lepidocyrtus lanuginosus* have a white opaque appearance and the eggs of *Isotomurus palustris* are bright yellow. During further development of the eggs those of some species become coloured (see Table 10).

(to be continued on page 161!)